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Antioxidant activity of sesame cake extract

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Abstract

Sesame cake was extracted with methanol to obtain a crude antioxidant extract. The qualitative and quantitative analysis of antioxidants/lignans present in the extract was carried out by reverse phase high performance liquid chromatography (HPLC) using a C_{18} column. In this study, sesame cake was also subjected to successive extractions with solvents of differing polarity to get a purified antioxidant extract with higher antioxidant content and better activity. The antioxidant activity was evaluated using the β-carotene bleaching method, linoleic acid peroxidation method and free radical scavenging assay, using 2,2-diphenyl-1-picryl hydrazyl radical (DPPH·). Results showed that crude extract was effective at 100 and 200 ppm levels and comparable with butylated hydroxy toluene (BHT) at 200 ppm, whereas purified extract showed comparable or better activities at 5, 10, 50, 100 and 200 ppm levels.

Keywords: Antioxidant activity; Sesame cake extract Thiocyanate method; DPPH assay; β-Carotene bleaching method

1. Introduction

Lipid oxidation is an important chemical change that lowers the nutritional quality of food. The primary and secondary products of lipid oxidation are detrimental to health. In the body, excess production of free radicals affects lipid cell membranes to produce lipid peroxides and reactive oxygen species (ROS) which lead to many biological changes, such as DNA damage, ageing, heart disease and cancer (Labuza, 1971). The addition of antioxidants is a method for increasing shelf-life of lipids and lipid-containing foods. Antioxidants are also of immense interest to health professionals as they may help to protect the body against damage caused by ROS (Shahidi, Janitha, & Wanasandura, 1992). Synthetic antioxidants, such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT), have restricted use in food as they are reported to be carcinogenic (Ito, Fukushima, & Tsuda, 1986). Some countries, such as Japan and Europe, have not permitted the use of tertiary butyl hydroquinone (TBHQ), the most potent synthetic food antioxidant and other countries may ban it (Shahidi, 1997) in the future. Therefore, the search for natural antioxidants has been greatly intensified in recent years.

Sesame has long been regarded, in the orient, as a health food, which increases energy and prevents ageing (Namiki, 1995). Sesame oil is highly resistant to oxidative deterioration, even though it is unsaturated. The stability of sesame oil is due to the presence of endogenous antioxidants, sesamin, sesamolin and sesamol. Sesamin is reported to possess in vivo hypocholesterolemic activity and suppressive activity against chemically induced cancer (Sugano et al., 1990; Yamashita, Lizuka, Imai, & Namiki, 1995). Sesame cake, a by-product of the oil industry, is currently used as a cattle feed. However, no studies have been conducted to investigate the antioxidant activity of sesame cake extracted with methanol. The present study was undertaken to evaluate the antioxidant potential of sesame cake extract and to utilize it as a substitute for synthetic antioxidants. The antioxidant effects of crude and purified extracts of sesame cake have been evaluated using in vitro peroxidation models and a radical scavenging method. The study also attempts to quantify the antioxidant compounds present in sesame cake extract.

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2. Materials and methods

2.1. Materials

Two popular brands of Sesame cake, namely, 'Idhayam' brand, manufactured by M/s.V.V.V. & Sons, Virudhu Nagar, Tamil Nadu, India and 'O.M.S' brand, manufactured by M/s. O.M.S. Mills, Virudhunagar, Tamil Nadu, India, were purchased from local markets. Samples of red, brown and white varieties of sesame (*Sesamum indicum*, L.) seeds, mixed and pressed for commercial extraction, were also collected from the same oil mills. HPLC grade solvents were from Merck, India. Standard sesamol was obtained from Sigma chemicals, USA. Sesamin and sesamolin were isolated and purified from sesame oil (Soliman, EI-Sawy, Fadel, & Osman, 1985). Lignan glycosides, namely, sesaminol diglucoside and triglucoside were separated by preparative HPLC.

2.2. Extraction

2.2.1. General

The details of extraction, purification, compositional analyses and activity of the antioxidant extract from sesame seeds/cake are part of the patent proposal submitted by authors through CSIR, India. (US Patent Application No. 60/404.004 August, 2001).

2.2.2. Crude extract

Sesame cake was dried and powdered. Ten grams was extracted with 150 ml methanol for 16 h in a soxhlet extractor. The extract was filtered and concentrated using a vacuum evaporator (Heidolph, Germany). The residue was weighed and redissolved in 100 ml methanol and stored under refrigeration until further analysis.

2.2.3. Purified extract

Commercial sesame cake was dried and powdered, using a steel mortar and pestle. One hundred grams of the sample was initially extracted with hexane (three times with a total of 1.5 l of hexane) at room temperature. The defatted residue was washed with distilled water (three times with a total of 1.5 l of distilled water) to remove soluble sugars and proteins and dried below 70 °C. Ten grams of the above purified residue was extracted with 150 ml methanol for 16 h in a soxhlet extractor. The extract was filtered, solvent removed under vacuum/N_2 flow to dryness, weighed and the residue redissolved in 100 ml of methanol to give an antioxidant extract of known concentration and stored in refrigeration until analyzed.

2.3. Total phenolic content

Total phenolic content (TPC) of the extract was measured by method (9.110) of the Association of Official Analytical Chemists AOAC (1984). Sesame cake extract (0.1 ml) was accurately transferred to a 100-ml volumetric flask containing 75 ml distilled water. Five milliliters of Folin–Ciocalteu reagent and 10 ml saturated sodium carbonate solution were added to the flask and diluted to 100 ml with distilled water. The mixture was then shaken for 1 min and allowed to stand at room temperature for 30 min. Absorbance of the solution was measured in a Shimadzu (model UV-160A) UV–Visible spectrophotometer at 760 nm. The experiment was repeated thrice and the mean value taken.

2.4. Analysis of lignans by HPLC

HPLC analysis was carried out in a Shimazdu binary system with LC-10 AD model pump, a 7125 model Rheodyne injector fitted with a 20 μl sample loop, a SPD-10A UV–Visible detector, with a C-R7Ae plus integrator for data acquisition, analysis and display. The analysis was carried out using a Waters μ-Bondapak C_{18} column (4.6 mm i.d × 25 cm) in the reverse phase connected through a guard column of C_{18} (Supelco). The mobile phase used was methanol:water (70:30) with a flow rate of 1 ml/min. The UV–Visible detector was set at 290 nm.

Standard sesamol was dissolved in methanol and solutions of different concentrations in the range of 0.2–1 μg/20 μl MeOH were prepared and injected into the HPLC to check the linearity between concentration and peak areas, and a response factor was calculated. Standard solutions of sesamin and sesamolin were also prepared by dissolving the required amount in methanol and suitable concentrations were injected to find the RT (retention time) values. Quantifications of lignans and lignan glucosides were done using these calibration factors. For separation and quantification of glycosides, preparative HPLC was employed, using a C_{18} column (20 mm i.d. × 25 cm) and the above solvent system at a flow rate of 10 ml/min. The respective fractions collected were freeze-dried and checked for purity by analytical HPLC.

2.5. Antioxidant activity by the β-carotene bleaching method

The antioxidant activities of extracts (crude and purified) were evaluated by the β-carotene-linoleate model system (Hidalgo, Fernadez, Quilhot, & Lissi, 1994; Jayaprakasha, Singh, & Sakariah, 2001). β-Carotene (0.2 mg), 20 mg of linoleic acid and 200 mg of Tween 20 were mixed in 0.5 ml chloroform and the solvent evaporated under vacuum. The resulting mixture was diluted with 50 ml oxygenated distilled water. To 4 ml of this emulsion, 0.2 ml of test samples in ethanol was added. BHT was used for comparative

purposes. A solution with 0.2 ml of ethanol and 4 ml of the above emulsion was used as control. The tubes were covered with aluminium foil and were maintained at 50 °C in a water bath. Absorbance was taken at zero time ($t = 0$) and after every 15 min. Measurement of absorbance was continued until the color of β-carotene disappeared in the control reaction ($t = 120$ min). The antioxidant activity of extracts was based upon three different parameters, namely antioxidant activity (A_A), the oxidation rate ratio (R_{OR}) and antioxidant activity coefficient (C_{AA}).

Antioxidant activity (A_A) was determined as percent inhibition relative to control sample

$$A_A = [(R_{control} - R_{sample})/(R_{control})] \times 100,$$

where $R_{control}$ and R_{sample} represent the bleaching rates of β-carotene without and with the addition of antioxidant, respectively. Degradatioin rates (R_D) were calculated according to first-order kinetics:

$$R_D = \ln(A_t/A_x) \times 1/t,$$

where ln is natural log, A_t is the initial absorbance at 470 nm at $t = 0$ and A_x is the absorbance at 470 nm at $t = 10$, 20, 30 min.

The oxidation rate ratio (R_{OR}) was calculated by

$$R_{OR} = R_{sample}/R_{control},$$

where R_{sample} and $R_{control}$ are as described earlier.

The antioxidant activity coefficient (C_{AA}) was calculated using:

$$C_{AA} = [(A_{s_{(120)}} - A_{c_{(120)}}/A_{c_{(0)}} - A_{c_{(120)}})] \times 1000,$$

where $A_{s_{(120)}}$ is the absorbance of the sample containing antioxidant at $t = 120$ min, $A_{c_{(120)}}$ is the absorbance of the control at $t = 120$ min and $A_{c_{(0)}}$ is the absorbance of the control at $t = 0$ min.

2.6. Linoleic acid emulsion system–thiocyanate method

Antioxidant activity of sesame cake extract was also evaluated using the thiocyanate method (Haraguchi, Hashimoto, & Yagi, 1992; Yen & Hsieh, 1998). The reaction mixture consisted of 0.28 g linoleic acid, 0.28 g of Tween 20 and 50 ml of phosphate buffer (0.2 M, pH 7.0). To 2.5 ml of the above linoleic acid emulsion, 0.5 ml of test sample and 2.5 ml of phosphate buffer (0.2 M, pH 7.0) were added and incubated at 37 °C for 120 h. The mixture prepared, as above, without test sample was the control. The readings were taken after each 24 h. The mixture (0.1 ml) was taken and mixed with 5.0 ml of 75% ethanol, 0.1 ml of 30% ammonium thiocyanate and 0.1 ml of 20 mM ferrous chloride in 3.5% HCl and allowed to stand at room temperature. Precisely 3 min after the addition of ferrous chloride to the reaction mixture, the absorbance at 500 nm was measured.

2.7. Linoleic acid system–thiocyanate method

Autoxidation of linoleic acid was carried out by using the following method (Choi, Chung, Jung, Park, & Yokozawa, 2000). Different amounts of samples dissolved in 0.1 ml of ethanol (100, 200 μg/ml) were added to a reaction mixture in a screw-cap vial. Each reaction mixture consisted of 2.5 ml of 0.02 M linoleic acid in ethanol and 2.0 ml of 0.2 M phosphate buffer (pH 7.0). The vial was incubated in an oven at 40 °C. At regular intervals during incubation, a 0.1 ml aliquot of the mixture was diluted with 4.0 ml of 75% ethanol, followed by the addition of 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance at 500 nm was measured. Solution without added samples were used as blanks.

2.8. Free radical scavenging activity using DPPH radical

To evaluate the free radical scavenging activity, the extracts were allowed to react with a stable free radical, 2,2-diphenyl-1-picryl hydrazyl radical (DPPH·) (Brand Williams, Cuvelier, & Berset, 1995; Sanchez-Moreno, Larrauri, & Saura-Calixto, 1998). Diluted cake extract (0.1 ml) was added to 3.9 ml DPPH· solution in methanol (0.025 g/litre). The reduction of (DPPH·) was followed by monitoring the decrease in absorbance at 515 nm until the reaction reached a steady state. The reaction time was 30 min.

(DPPH·) concentration in the medium at different time intervals was calculated from the calibration curve drawn using the following equation:

$$A_{515\ nm} = 2935.68(DPPH^{\cdot})_t - 2.18 \times 10^{-3}.$$

The percentage of remaining (DPPH·) at the steady state was

$$\%DPPH_{REM} = (DPPH^{\bullet})_T/(DPPH^{\bullet})_{T=0}$$

The amount of sample needed to decrease the initial DPPH concentration by 50%, EC_{50}, was calculated graphically. The anti-radical power (ARP) of extract calculated as

$$ARP = 1/EC_{50}$$

All the above activity studies were carried out with triplicate sets and, from these, duplicate samples were drawn for estimation. The mean of six determinations was taken for further calculations.

2.9. Statistical analysis

The Student's t test was used to compare the data and all tests were considered statistically significant at $P < 0.05$.

3. Results and discussion

The effectiveness of various solvent systems and extraction conditions on the yield of sesamol and lignan content from sesame seed, oil and cake was initially studied. Both polar and non-polar solvents were used for soxhlet and cold extraction. Methanol was found to be the most efficient solvent for extracting the antioxidant compounds, especially sesamol and the lignans. The amounts of these were quantified by HPLC analysis, using a reverse phase C_{18} column. The lignan profiles and contents of seed and oil are given in Table 1 and those of cake in Table 2. From the values, it is clear that, after oil extraction the cake still retains the lignans. Hence, for further studies, sesame cake, a cheaper source and by-product, was used. Removal of oil by hexane before methanolic extraction of seed/cake improved the extraction efficiency of lignans. The extraction conditions and purification steps were optimized using total phenolic content (TPC) and lignan content values. TPC was determined in comparison with standard gallic acid and the results expressed in terms of ppm (μg/g of extract). The TPC values for the crude and purified extracts were 1709 ± 2.40 and 5438 ± 11.9 ppm, respectively. Even though there was an increase in TPC values after purification, it did not give much correlation with lignan content. This may be due to the fact that different phenolic compounds have different responses in the Folin method (Kahkonen et al., 1999).

Fig. 1 shows the HPLC profile of sesame cake extract in methanol. Total lignan content was calculated from the quantities of sesamol, sesamin and sesamolin by HPLC analysis (Table 2). Lignan glycosides were calculated from sesaminol triglucoside and diglucoside contents. Peaks were identified by co-injection with standard compounds. The quantification of lignans present in methanolic extract of sesame cake and the

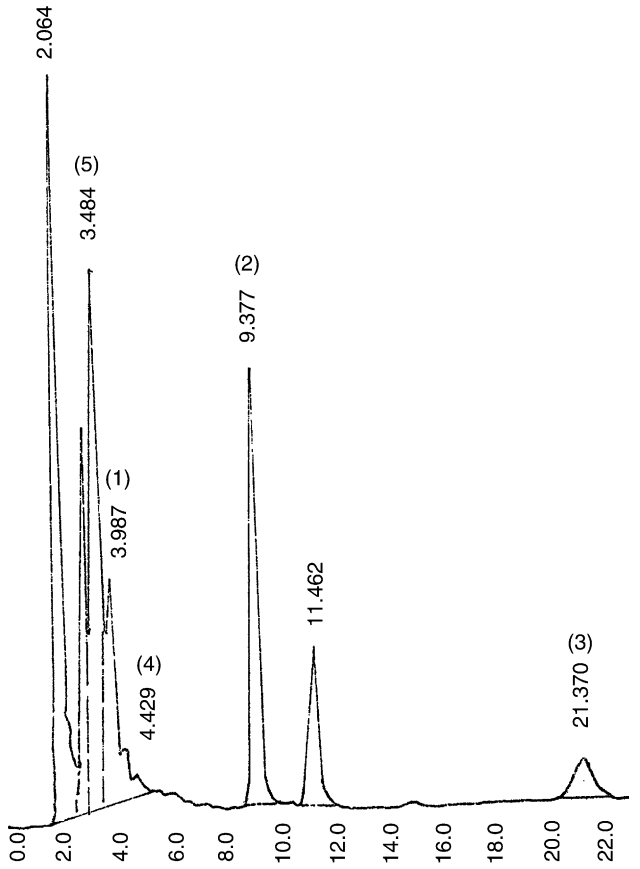

Fig. 1. HPLC profile of sesame cake extract. (1) Sesamol; (2) sesamin; (3) sesamolin; (4) sesaminol diglucoside; and (5) sesaminol triglucoside (see conditions in the text).

Table 1
Lignan contents (ppm) of seed and oil

Sample	Sesamol	Sesamin	Sesamolin	Total lignan content
White seed extract in methanol	3834 ± 6.2	3993 ± 4.1	2054 ± 4.7	9881 ± 6.4
Red seed extract in methanol	2092 ± 5.2	3610 ± 7.9	2941 ± 1.8	8643 ± 9.6
Black seed extract in methanol	4306 ± 4.9	2037 ± 3.8	3563 ± 4.1	$99055 \pm .9.0$
Oil/white seed	Trace	4278 ± 1.8	2740 ± 2.7	7018 ± 2.5
Oil/red seed	Trace	4193 ± 4.2	1821 ± 3.7	6014 ± 3.0
Oil/Black Seed	Trace	1154 ± 3.9	502 ± 6.6	1653 ± 4.9

Table 2
Lignan contents (ppm) of sesame cake extract

Sample	Crude extract	Purified extract
Sesamol	2359 ± 12.3	22677 ± 2.5
Sesamin	4431 ± 4.1	105893 ± 217
Sesamolin	939 ± 8.9	12504 ± 4.0
Total lignans	7729 ± 22.3	141074 ± 272
Sesaminol triglucoside	5061 ± 33.9	6792 ± 23.9
Sesaminol diglucoside	1756 ± 128	6506 ± 98.7
Total glucoside	6817 ± 214	13298 ± 210
TPC (ppm)	1709 ± 29.4	5438 ± 43.7

presence of sesamol in sesame cake extract are reported for the first time. Linearity of analysis was checked with the help of standard sesamol. After purification, there was a significant increase in the content of antioxidants/lignans, by approximately 15–20% (Table 2). In the purification steps, washing with water helped to remove the sugars and soluble proteins, thus resulting in enhanced antioxidant content, better activity and dispersibility of the final extract. Recently, Shyu and Hwang (2002) have reported lignans of defatted, sesame meal extract. However, the lignan content of our purified extract is much higher than the above and is characterized by the presence of sesamol in it.

The antioxidant activities of sesame cake extracts were evaluated by the β-carotene bleaching method, in which the oxidation of β-carotene in the presence of linoleic acid takes place. Fig. 2 shows the effect of extracts in comparison with BHT. Crude and purified extracts were tested at the 100 and 200 ppm levels and BHT at 200 ppm. Since the maximum permissible level allowed for synthetic antioxidants for food use is 200 ppm, this concentration was used for reference antioxidant (BHT). It can be seen that both crude and purified extracts showed appreciable antioxidant activity. Crude extract showed 41.7% inhibition at 100 ppm and 46.6% inhibition at 200 ppm concentration while BHT showed 45.6% inhibition at 200 ppm concentration. The activity of crude extract was comparable to BHT at 200 ppm.

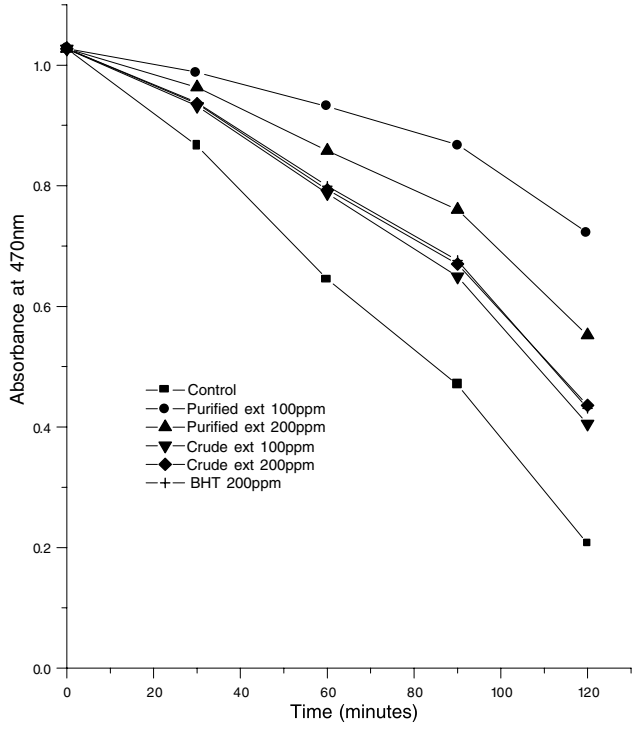

Fig. 2. Antioxidant activity of sesame extracts and BHT by the β-carotene bleaching method.

Table 3
Dose (ppm)–response of antioxidant activity for the sesame cake extracts by β-carotene bleaching method

Concentration	A_A[a]	R_{OR}[b]	C_{AA}[c]
5 ppm (purified extract)	63.0	0.369	602
10 ppm (purified extract)	67.1	0.328	662
50 ppm (purified extract)	81.6	0.183	905
100 ppm (purified extract)	77.9	0.220	629
200 ppm (purified extract)	61.1	0.388	421
BHT 200 ppm	45.6	0.543	274
100 ppm (crude extract)	41.7	0.583	242
200 ppm (crude extract)	46.6	0.540	280

[a] Antioxidant activity index.
[b] Oxidation rate ratio.
[c] Antioxidant activity coefficient.

Purified extracts showed better activity than crude extracts. The dose–response relationship for the purified extract is shown in Table 3. The activity indices at 5 and 10 ppm were 63.0 and 67.1, respectively. The activity index at 50 ppm was 81.6 while that for BHT at 200 ppm was 45.6. After 60 min, the antioxidant activity index for purified extract was double that of BHT, while crude extract showed comparable results with that of BHT. The activity of purified extract at 100 ppm is better than at 200 ppm. Linoleic acid hydroperoxides attack the β-carotene molecule and, as a result, it undergoes rapid decolorization. The corresponding decrease in absorbance can be monitored spectrophotometrically. The presence of antioxidant extracts can hinder the extent of β-carotene bleaching by acting on the free radicals formed in the system (Jayaprakasha et al., 2001).

The data for oxidation rate ratio and activity coefficient support the antioxidant activity index. Oxidation rate ratio bears an inverse relationship with antioxidant activity index. The oxidation rate ratios for purified extracts were lower than that for crude extracts and BHT. Activity coefficient increases directly with the increase in value of the antioxidant activity index.

The antioxidant effects of sesame extracts prevent the peroxidation of linoleic acid, as measured by thiocyanate method. Both the linoleic acid system and emulsion system were evaluated to understand the efficacy in homogeneous and heterogeneous systems, results of which are shown in Figs. 3 and 4. Absorbance of control was increased due to the oxidation of linoleic acid hydroperoxides, which decompose to many secondary oxidation products (Hua-Ming, Koji, Fumio, & Nokihara, 1996). These oxidized products react with ferrous sulphate to form ferric sulphate which, on further reaction with ammonium thiocyanate, forms the ferric thiocyanate red color. Antioxidants can slow down the peroxidation of linoleic acid; hence, the ferric thiocyanate formation will be slow.

Real food systems generally consist of multiple phases in which lipid and water coexist with some emulsifier. Hence an antioxidant assay, using a heterogeneous

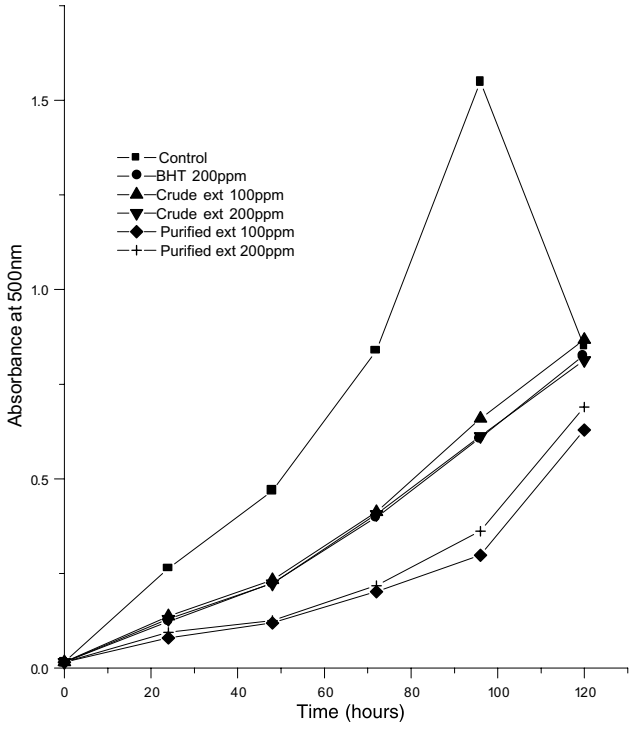

Fig. 3. Antioxidant activity of sesame extracts and BHT by the thiocyanate method–linoleic acid emulsion system.

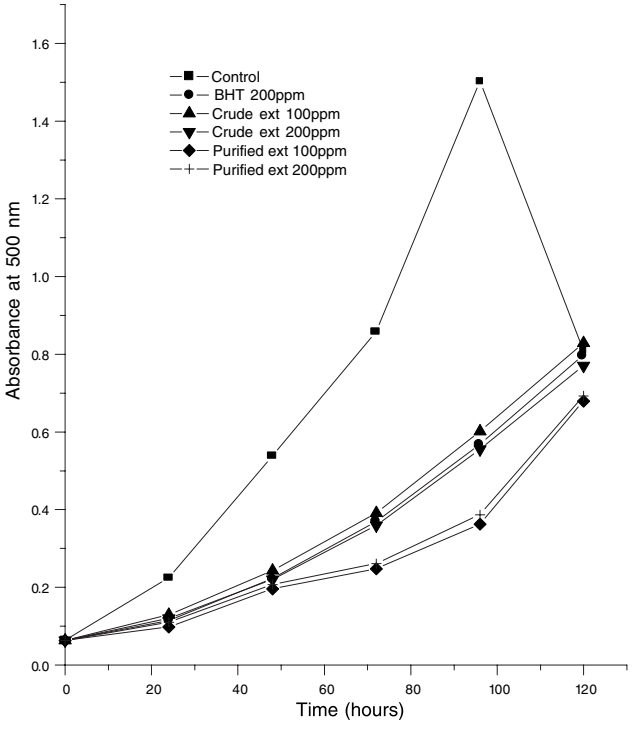

Fig. 4. Antioxidant activity of sesame extracts and BHT by the thiocyanate method–linoleic acid system.

system such as oil in water emulsion, is also required. Therefore the current assay includes both linoleic acid and linoleic acid emulsion systems which represent homogeneous and heterogeneous systems (Osawa & Namiki, 1981). The linoleic acid system can be correlated with a homogeneous system or bulk oil phase system. The linoleic acid emulsion system can be simulated with the biological lipid system or with food/fat emulsions. Extracts showed antioxidant activity in both methods. The percentage inhibitions for crude extract at 100, 200 ppm, purified extract at 100, 200 ppm and BHT at 200 ppm were 57%, 61%, 81%, 77% and 61% in the linoleic acid emulsion system and 62%, 63%, 76%, 74% and 62% in the linoleic acid system, respectively. Purified extract showed more protective effect than crude extract. This may be due to the high content of lignans and partial removal of interfering compounds, such as fat.

DPPH·, a stable free radical with a characteristic absorption at 515 nm, was used to study the radical scavenging effects of extracts. As antioxidants donate protons to this radical, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. The EC_{50} value for crude extract was 150×10^3 μg/ml and that for purified extract was 5.49×10^3 μg/ml. The corresponding anti-radical power (ARP) values were 0.648×10^{-5} and 17.7×10^{-5}, respectively. There is significant increase in free radical scavenging power of purified extract (Figs. 5 and 6).

In the present study antioxidant activity was evaluated by different model systems. The β-carotene bleaching method and thiocyanate methods were peroxidation models, where inhibition of peroxidation is taken as the index of activity. The DPPH· assay evaluated the free radical scavenging power of extract.

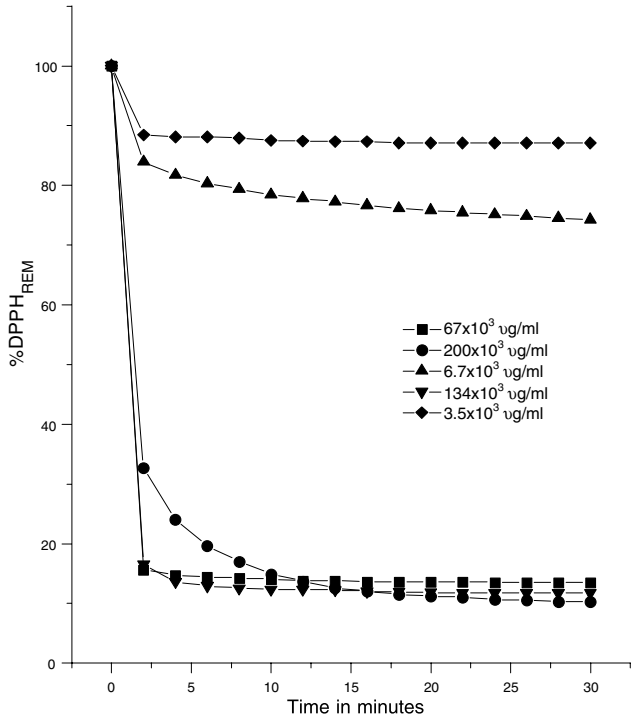

Fig. 5. DPPH scavenging effect of crude sesame cake extract.

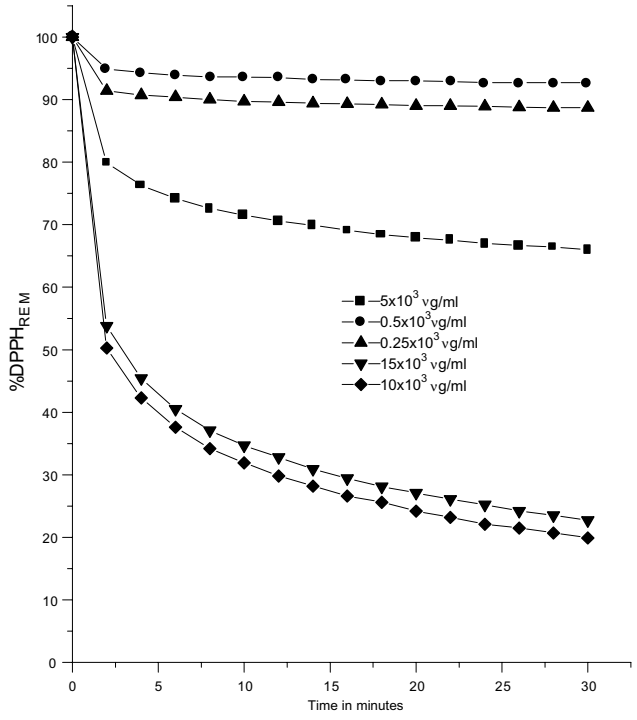

Fig. 6. DPPH scavenging effect of purified sesame cake extract.

The antioxidative activity of extracts is concentration-dependent. Purified extract is more effective at 50 and 100 ppm and the effect is more than that of the synthetic antioxidant, BHT, at 200 ppm. The crude extract showed activity comparable to that of BHT. The results of the present study indicate the presence of compounds possessing high antioxidant activity in sesame cake extract. By purification, the antioxidant content and dispersibility of extract increased, which resulted in higher activity.

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